



INVESTOR IN PEOPLE



PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

REC'D 13 JAN 2005

WIPO PCT

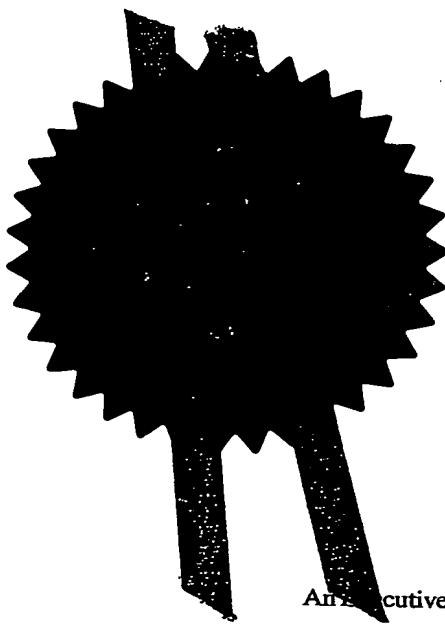
The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed

Dated 31 December 2004

BEST AVAILABLE COPY



1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form.)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

Fee: £0

08DEC03 E957485-4 D01631

46684.GB01/NT P01/7700 0.00-0328323.1

1. Your reference

2. Patent application number
(The Patent Office will fill in this part)

3. Full name, address and postcode of the or of each applicant (*underline all surnames*)

08208761 002

Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of incorporation

05 DEC 2003

0328323.1

Cambridge Biotechnology Limited
PO Box 230
Cambridge
CB2 1XJ
UNITED KINGDOM

4. Title of the invention

Synthesis of 2-substituted Adenosines

5. Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
WC1X 8PL

Patents ADP number (*if you know it*)

91001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (*if you know it*) the or each application number

Country

Priority application
(*if you know it*)

Date of filing
(day/month/year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day/month/year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document.

Continuation sheets of this form	0
Description	12
Claim(s)	5
Abstract	0
Drawing(s)	0

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent (<i>Patents Form 7/77</i>)	0
Request for preliminary examination and search (<i>Patents Form 9/77</i>)	0
Request for substantive examination (<i>Patents Form 10/77</i>)	0
Any other documents <i>(please specify)</i>	0

11.

I/We request the grant of a patent on the basis of this application

Signature

Date
4 December 2003

Freddie e Grose

12. Name and daytime telephone number of person to contact in the United Kingdom

Neil Thornton
01223 360350

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

46684.GB01

Synthesis of 2-substituted Adenosines

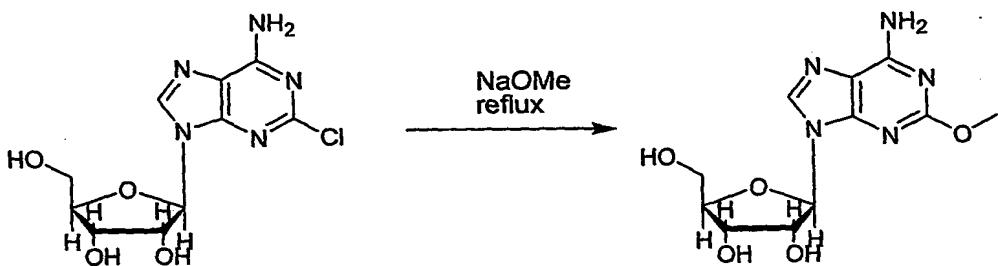
This invention relates to synthesis of 2-substituted adenosines, such as spongosine (2-methoxyadenosine), and synthesis of intermediates for use in the synthesis of such compounds.

The natural product spongosine was first isolated from a sponge, *Cryptotethia crypta*, collected off the Florida coast in 1945 (Bergmann and Feeney, J.Org. Chem. 1951, 16, 981; Ibid 1956, 21, 226). Spongosine was considered an unusual nucleoside in that it was not only the first methoxypurine to be found in nature but also one of the first O-methyl compounds to be isolated from animal tissues.

Several methods of synthesis of spongosine have been reported. One of the first of these to be published was by Bergmann and Stempien (J. Org. Chem. 1957, 22, 1575) in which spongosine was formed via coupling of chloromericuric 2-methoxyadenine to 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride. This simple coupling reaction provided a crude yield of spongosine of 31% which was then recrystallised from hot water to provide spongosine which exhibited a melting point of 191-191.5°C and an optical rotation of -43.5° (NaOH).

A variation on this theme was employed by Ojha *et al.* (Nucleosides and Nucleotides (1995, 14, 1889) who initially coupled 2-ethylthioadenine with a suitably protected ribose. Subsequent adjustments of the protecting groups and oxidation gave a substrate which was reacted with sodium methoxide to yield spongosine in a yield of 87% for the final step. The purity of the target spongosine after column chromatography, was proved by both elemental analysis and melting point (189-190°C).

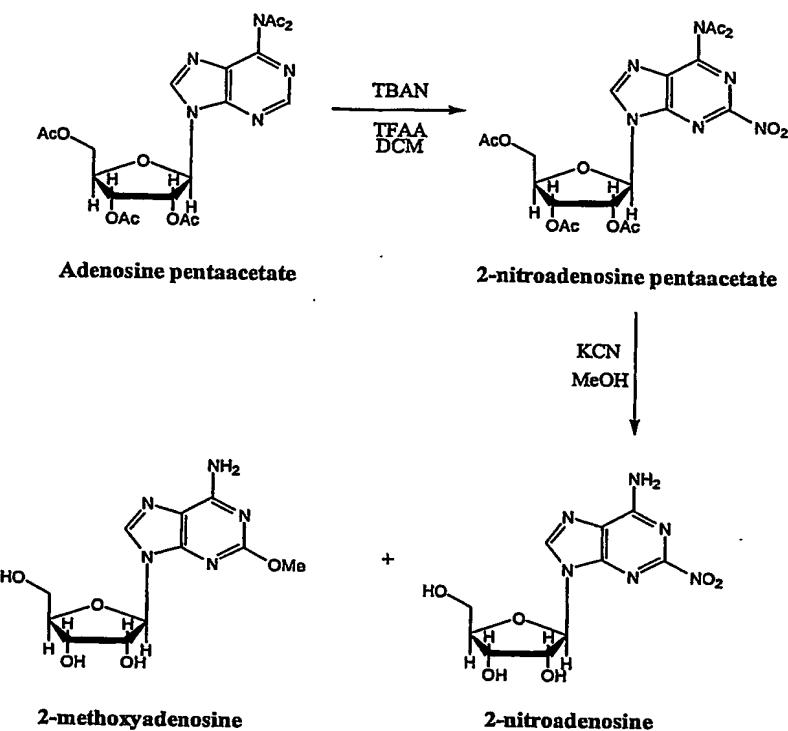
One of the most common methods of preparation of spongosine is via displacement of a 2-substituted chlorine atom by methoxide:



This methodology has been successfully applied by a number of groups to provide spongiosine in varying yields and purity: Schaeffer *et al.*, J. Am. Chem. Soc. 1958, 80, 3738 (35% yield, mpt. 190-192°C); Bartlett *et al.*, J. Med. Chem. 1981, 24, 947 (yield and purity not quoted); Sato *et al.*, Synth. Proceed. Nucleic Acid Chem. 1968, 1, 264. However, this method suffers from the disadvantage that the 2-chloroadenosine starting material is difficult to synthesise, and consequently is expensive to produce.

Spongiosine was reported by Cook *et al.* (J. Org. Chem. 1980, 45, 4020) as a by-product in the methylation reaction of isoguanosine by methyl iodide. Both the desired 1-methylisoguanosine and the spongiosine were obtained in poor crude yields (19 and 30% respectively). The crude spongiosine fragment was first purified by column chromatography on silica gel (eluent: chloroform/methanol) and then recrystallised from water to provide a sample which melted between 189-192°C (7% yield pure).

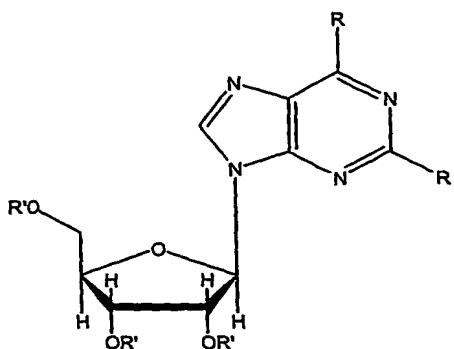
Paymaneh *et al* (Tetrahedron Letters 41 (2000) 1291-1295) and Wanner *et al* (Bioorganic & Medicinal Chemistry Letters 10 (2000) 2141-2144) describe formation of spongiosine as a significant by-product in the synthesis of 2-nitroadenosine by treatment of 2-nitroadenosine pentaacetate with potassium cyanide in methanol. The 2-nitroadenosine was obtained in only 10% yield, and spongiosine in 47% yield (Paymaneh *et al*). The 2-nitroadenosine pentaacetate was produced by nitration of adenosine pentaacetate with tetrabutylammonium nitrate/trifluoroacetic anhydride (TBAN/TFAA):



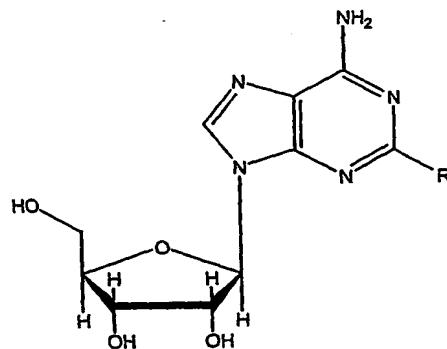
A disadvantage of this method is that the spongiosine is not produced in high yield or purity. A further disadvantage is that it involves use of the toxic reagent potassium cyanide.

It is desired, therefore, to provide alternative methods of synthesis of spongiosine and other 2-substituted adenosines, and of intermediates for use in the synthesis of these compounds. It is also desired to improve the yield and purity of the 2-substituted adenosines and intermediates obtained.

According to a first aspect of the invention there is provided a method of synthesis of a compound of formula I which comprises converting a compound of formula II to a compound of formula I:



II



I

wherein:

R is C₁₋₆ alkoxy (straight or branched), a phenoxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy), or a benzoyl group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy;

R' = H, or a protecting group

Preferably R is methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, phenoxy, or benzoyl.

Preferably the compound of formula I produced is isolated.

In some preferred embodiments of the invention R' is H, and the compound of formula II is aminated to form the compound of formula I. This may be achieved, for example by heating the compound of formula II in a solution of ammonia (for example upto 80°C) and then cooling the solution to precipitate the compound of formula I. Preferably an aqueous solution of ammonia is used, although ammonia in methanol or ethanol may alternatively be used. Preferably the precipitate is then isolated, for example by filtration and washing.

Preferably the compound of formula II is 2,6-dimethoxy adenosine, and the compound of formula I is spongiosine. A preferred method of converting 2,6-dimethoxy adenosine to

spongosine and isolating the spongiosine produced is described in Step 5 of the Example below.

In other preferred embodiments of the invention R' is a protecting group. It is advantageous if the protecting group is removed under the same conditions that replace the R group at the 6-position of the purine component of the compound of formula II with an amino group. This allows the compound of formula II to be converted to the compound of formula I in a single reaction step. It is preferred that R' is a protecting group that can be removed by treatment with ammonia. Suitable protecting groups are acetyl and benzoyl.

Preferably methods of the first aspect of the invention further comprise converting triacetoxy 2-nitro-6-chloroadenosine to the compound of formula II.

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I which includes the step of converting triacetoxy 2-nitro-6-chloroadenosine to a compound of formula II.

There is also provided according to a further aspect of the invention a method of synthesis of a compound of formula II which comprises converting triacetoxy 2-nitro-6-chloroadenosine to the compound of formula II.

Preferably the compound of formula II produced is isolated.

Preferably triacetoxy 2-nitro-6-chloroadenosine is alkoxylated or benzoylated at the 2- and 6- positions to form the compound of formula II.

For embodiments of the invention in which the compound of formula II is 2,6-dimethoxyadenosine, preferably triacetoxy 2-nitro-6-chloroadensine is methoxylated at the 2- and 6- positions to form 2, 6-dimethoxy adenosine. This may be achieved, for example by contacting a solution of sodium methoxide in methanol with a solution of triacetoxy 2-nitro-6-chloroadenosine in dichloromethane (DCM) or chloroform.

An advantage of use of sodium methoxide/methanol as methoxylating reagent is that it is considerably less toxic than potassium cyanide/methanol used by Paymaneh *et al.*, and Wanner *et al.* Sodium methoxide/methanol also appears to give a higher yield of methoxylated product than potassium cyanide/methanol.

Preferably the 2, 6-dimethoxy adenosine is then isolated from the contacted solutions, for example by removing the methanol and DCM and purifying the 2, 6-dimethoxy adenosine by reverse phase column chromatography.

A preferred method of converting triacetoxy 2-nitro-6-chloroadenosine to 2, 6-dimethoxy adenosine and isolating the 2, 6-dimethoxyadenosine produced is described in Step 4 of the Example below.

Preferably methods of the first or further aspects of the invention further comprise converting triacetoxy 6-chloroadenosine to triacetoxy 2-nitro-6-chloroadenosine.

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I or a compound of formula II which includes the step of converting triacetoxy 6-chloroadenosine to triacetoxy 2-nitro-6-chloroadenosine.

Preferably the triacetoxy 2-nitro-6-chloroadenosine produced is isolated.

Preferably the compound of formula I is spongiosine, and the compound of formula II is 2,6-dimethoxy adenosine.

Preferably triacetoxy 6-chloroadenosine is nitrated at the 2-position to form triacetoxy 2-nitro, 6-chloroadenosine. Suitable nitrating reagents include tetrabutyl ammonium nitrate (TBAN), tetramethyl ammonium nitrate (TMAN) and sodium nitrate. For example a solution of triacetoxy 6-chloroadenosine may be contacted with a solution of TBAN and trifluoroacetic acid (TFAA), or TMAN and TFAA. Preferably a chlorinated solvent is used, such as DCM or chloroform.

Nitration of triacetoxy 6-chloroadenosine to triacetoxy 2-nitro-6-chloroadenosine using TBAN/TFAA in DCM is described in Paymaneh *et al.*, page 1292, lines 4-6 (although not in relation to synthesis of spongiosine). TBAN/TFAA is also used by Paymaneh *et al.* to nitrate adenosine pentaacetate in the method of synthesis of spongiosine disclosed in this document.

We have appreciated, however, that one of the principal reasons that spongiosine is not produced in high yield and purity by the method of Paymaneh *et al.* is that TBAN and other tetrabutyl ammonium (TBA) salts contaminate the 2-nitroadenosine pentaacetate intermediate and interfere with subsequent synthesis steps.

According to the invention, the yield and purity of the spongiosine product can be significantly improved if the amount of contaminating TMA salts is reduced. However, removal of these contaminants is problematic because they are amphiphilic and so cannot be completely removed by aqueous work-up.

We have found that the purity and yield of triacetoxy 2-nitro-6-chloroadenosine and subsequently produced 2, 6-dimethoxyadenosine and spongiosine is surprisingly significantly improved by trituration of the triacetoxy 2-nitro-6-chloroadenosine from isopropanol, or preferably ethanol, and washing with a mixture of water and ethanol to remove the TBA impurities.

We have appreciated that similar methods can be used to remove tetramethyl ammonium (TMA) impurities if tetramethyl ammonium nitrate (TMAN) is used as nitrating reagent instead of TBAN.

The TBA or TMA impurities are easier to remove from triacetoxy 2-nitro-6-chloroadenosine than from 2-nitroadenosine pentaacetate (used by Paymaneh *et al.*) because this latter compound decomposes in water. Thus, spongiosine can be synthesised more easily in high yield and purity by using a triacetoxy 6-chloroadenosine intermediate.

A preferred method of converting triacetoxy 6-chloroadenosine to triacetoxy 2-nitro-6-chloroadenosine and isolating the triacetoxy 2-nitro-6-chloroadenosine produced is described in Step 3 of the Example below.

We have appreciated that the above methods can be used to remove TBA or TMA impurities that contaminate compounds synthesised in other reactions by nitration of a substituted adenosine using TBAN or TMAN. The compounds may thereby be produced in higher purity, and the purity and yield of products produced by subsequent synthesis steps may be increased.

Thus, according to a further aspect of the invention there is provided a method of reducing the amount of TBA or TMA impurities contaminating a product formed by nitration of a substituted adenosine with TBAN or TMAN, which comprises triturating the product from isopropanol or ethanol, and washing the product with a mixture of water and ethanol.

There is also provided according to the invention a method of producing a nitrated substituted adenosine which comprises nitrating a substituted adenosine using TBAN or TMAN, and reducing the amount of TBA or TMA impurity contaminating the nitrated substituted adenosine.

Preferably the amount of TBA or TMA impurity is reduced by triturating the nitrated substituted adenosine from isopropanol or ethanol, and washing the triturated product with a mixture of water and ethanol.

In general, a minimum of three washes with water/ethanol has been found to be required to remove a large proportion of the TBA or TMA impurities. However, five washes are generally carried out to ensure as much TBA or TMA impurity is removed as possible.

Instead of trituration, it may be possible to use column chromatography or reverse phase chromatography to reduce the amount of TBA or TMA impurity present.

The invention also provides nitrated, substituted adenosines produced by such methods.

Preferably methods of the first or further aspects of the invention further comprise converting triacetoxy inosine to triacetoxy 6-chloroadenosine.

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I or a compound of formula II which includes the step pf converting triacetoxy inosine to triacetoxy 6-chloroadenosine.

Preferably the triacetoxy 6-chloroadenosine produced is isolated.

Preferably the compound of formula I is spongamine, and the compound of formula II is 2,6-dimethoxy adenosine.

Preferably triacetoxy inosine is chlorinated to form triacetoxy 6-chloroadenosine. This may be achieved, for example by contacting DMF and thionyl chloride with a solution of triacetoxy inosine in choloroform. Instead of chloroform, DCM may be used as a solvent. Instead of thionyl chloride, POCl_3 may be used as chlorinating reagent.

Preferably the triacetoxy 6-chloroadenosine is isolated from the contacted DMF, thionyl chloride, and triacetoxy inosine solution, for example by removal of the DMF, thionyl chloride, and chloroform, partitioning of the resulting residue between DCM and aqueous sodium bicarbonate, and washing of the separated organic phase with brine and drying over magnesium sulphate.

A preferred method of forming the triacetoxy 6-chloroadenosine from triacetoxy inosine, and isolating the triacetoxy 6-chloroadenosine produced is described in step 2 of the Example below.

Preferably methods of the first or further aspects of the invention further comprise converting inosine to triacetoxy inosine.

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I or a compound of formula II which includes the step of converting inosine to triacetoxy inosine.

Preferably the triacetoxy inosine produced is isolated.

Preferably the compound of formula I is spongosine, and the compound of formula II is 2,6-dimethoxy adenosine.

Preferably inosine is acetylated to form triacetoxy inosine. This may be achieved, for example by contacting a suspension of inosine and catalytic DMAP in MeCN with Et₃N and acetic anhydride to form a solution before contacting the solution with methanol

A preferred method of converting inosine to triacetoxy inosine and isolating the triacetoxy inosine produced is described in Step 1 of the Example below.

According to the invention there is also provided use of a compound of formula II, triacetoxy 2-nitro, 6-chloroadenosine, triacetoxy 6-chloroadenosine, triacetoxy inosine, or inosine in the synthesis of a compound of formula I.

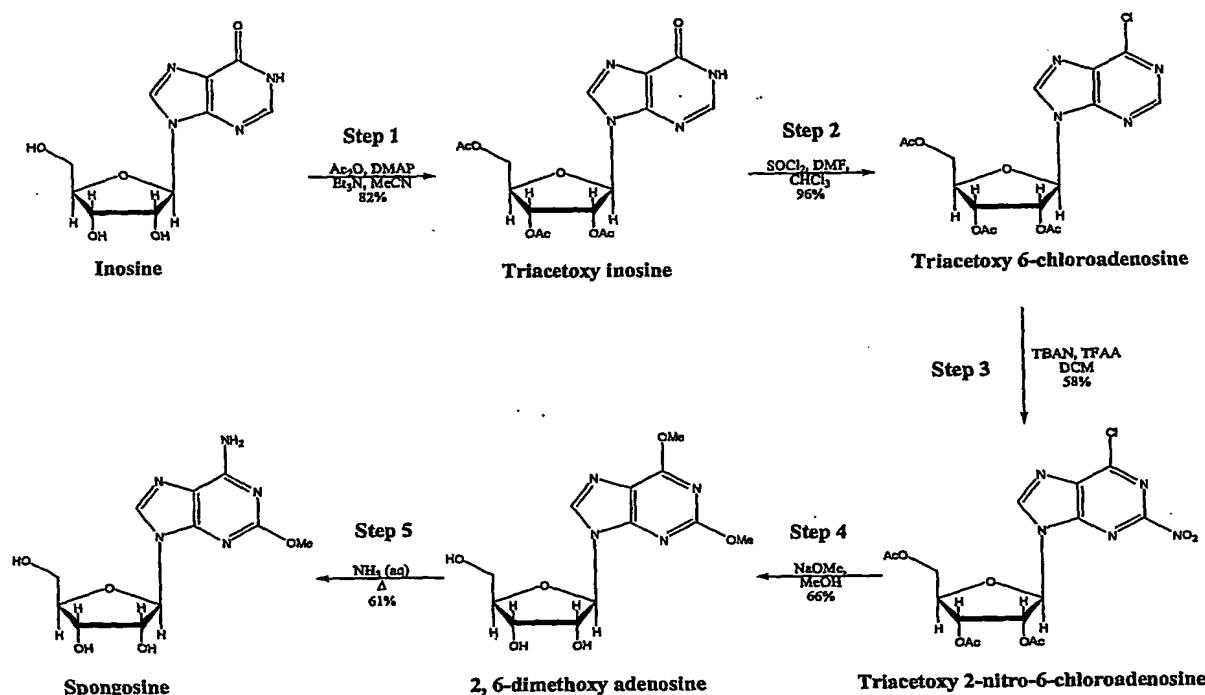
The invention further provides use of triacetoxy 2-nitro, 6-chloroadenosine, triacetoxy 6-chloroadenosine, triacetoxy inosine, or inosine in the synthesis of a compound of formula II.

Preferably the compound of formula I is spongosine and the compound of formula II is 2,6-dimethoxy adenosine.

Methods of the invention allow synthesis of compounds of 2-substituted adenosines and intermediates for use in the synthesis of 2-substituted adenosines in high yield and purity, and do not require use of toxic reagents such as potassium cyanide.

Embodiments of the invention are now described by way of example only with reference to the accompanying Scheme 1 which shows the synthesis of spongine from inosine.

Example



Scheme 1

Step 1

To a suspension of inosine (10g, 37.3mmol) and catalytic DMAP in MeCN (60mL) was added Et₃N (20mL, 143mmol) and acetic anhydride (12.5mL) and the resulting solution was stirred for 1h at ambient temperature before the addition of MeOH (5mL). After stirring for 5mins, the solution was concentrated *in vacuo* to yield a white solid which was washed with isopropyl alcohol to afford triacetoxy inosine (12.1g, 82%).

Step 2

To a solution of triacetoxy inosine (3.00g, 7.63mol) in CHCl₃ (25mL) was added DMF (1.80mL, 22.9mmol) and thionyl chloride (1.68mL, 22.9mmol) and the resulting solution was refluxed overnight before removal of the solvents *in vacuo*. The residue was then

partitioned between DCM and aq. NaHCO₃ and the separated organic phase was washed with brine and dried over MgSO₄ to afford triacetoxy 6-chloroadenosine as a pale yellow oil (3.03g, 96%).

Step 3

To a solution of TBAN (4.43g, 14.5mmol) in DCM (15mL) at 0°C was added TFAA (2.05mL, 14.5mmol) and the resulting solution was stirred for 5mins, before the addition of triacetoxy 6-chloroadenosine (4g, 9.7mmol) in DCM (20mL). The resulting brown solution was stirred for 2.5h before being quenched with aq. NaHCO₃, extracted into DCM and dried over MgSO₄. Purification *via* trituration from EtOH yielded triacetoxy 2-nitro, 6-chloroadenosine as a pale yellow solid which was washed with 1:1 EtOH/water to afford 2.57g, 58%.

Step 4

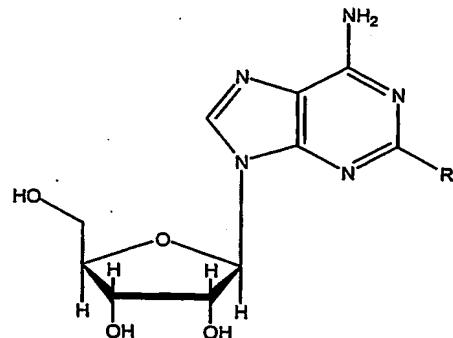
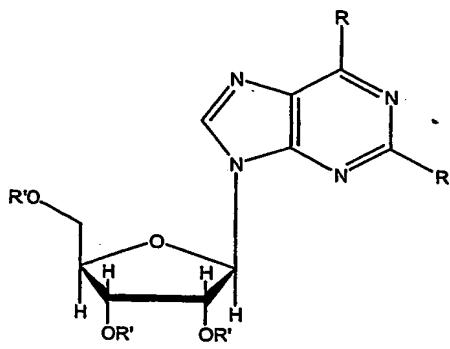
To a solution of NaOMe (590mg, 10.9mmol) in MeOH (10mL) was added dropwise a solution of triacetoxy 2-nitro, 6-chloroadenosine (1g, 2.19mmol) in DCM (10mL) and the resulting red solution was stirred overnight. The solvents were then removed *in vacuo* and the product was purified by reverse phase column chromatography (gradient 30-70% MeOH/water) to afford 2,6-dimethoxy adenosine as a white solid (447mg, 66%).

Step 5

A solution of 2,6-dimethoxy adenosine (697mg, 3.23mmol) in aq. NH₃ was heated in a sealed tube at 80°C for 26h. The solution was then cooled and the resulting white precipitate was filtered and washed with cold water to afford 2-methoxy adenosine (406mg, 61%).

Claims

1. A method of synthesis of a 2-substituted adenosine of formula I which comprises converting a compound of formula II to a compound of formula I:



wherein:

R is C₁₋₆ alkoxy (straight or branched), a phenoxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy), or a benzoyl group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy);

R' = H, or a protecting group

2. A method according to claim 1, wherein R = methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, phenoxy, or benzoyl.

3. A method according to claim 1 or 2, wherein R' is a protecting group that can be removed under conditions that replace the R group with an amino group at the 6-position of the purine component of the compound of formula II.

4. A method according to claim 3, wherein the compound of formula II is converted to the compound of formula I in a single reaction step.

5. A method according to any preceding claim, wherein the protecting group is acetyl or benzoyl, and the compound of formula I is converted to spongine by treatment with ammonia.
6. A method according to claim 1 or 2, wherein R' is H, and the compound of formula II is aminated to form the compound of formula I.
7. A method according to claim 6, wherein the compound of formula II is aminated by heating the compound in a solution of ammonia and then cooling the solution to precipitate the compound of formula I.
8. A method according to any preceding claim, which further comprises isolating the compound of formula I produced.
9. A method according to any preceding claim, which further comprises converting triacetoxy 2-nitro-6-chloroadenosine to a compound of formula II.
10. A method of synthesis of a compound of formula II which comprises converting triacetoxy 2-nitro-6-chloroadenosine to the compound of formula II.
11. A method according to claim 9 or 10, wherein triacetoxy 2-nitro-6-chloroadenosine is alkoxylated or benzoylated to form the compound of formula II.
12. A method according to claim 11, wherein triacetoxy 2-nitro-6-chloroadenosine is methoxylated using sodium methoxide in methanol as methoxylating reagent.
13. A method according to any of claims 9 to 12, which further comprises isolating the compound of formula II produced.
14. A method according to any of claims 9 to 13, which further comprises converting triacetoxy 6-chloroadenosine to triacetoxy 2-nitro-6-chloroadenosine.

15. A method according to claim 14, wherein triacetoxy 6-chloroadenosine is nitrated to form triacetoxy 2-nitro-6-chloroadenosine.
16. A method according to claim 14 or 15, which further comprises isolating the triacetoxy 2-nitro-6-chloroadenosine produced.
17. A method according to any of claims 14 to 16, wherein triacetoxy 6-chloroadenosine is nitrated to triacetoxy 2-nitro-6-chloroadenosine using tetrabutyl ammonium nitrate (TBAN) or tetramethyl ammonium nitrate (TMAN) as nitrating reagent.
18. A method according to claim 17, which further comprises reducing the amount of tetrabutyl ammonium (TBA) or tetramethyl ammonium (TMA) impurities contaminating the triacetoxy 2-nitro-6-chloroadenosine.
19. A method according to claim 18, wherein the amount of TBA or TMA impurities is reduced by triturating the triacetoxy 2-nitro-6-chloroadenosine from isopropanol or ethanol, and washing the triturated triacetoxy 2-nitro-6-chloroadenosine with a mixture of water and ethanol.
20. A method according to any of claims 14 to 19, which further comprises converting triacetoxy inosine to triacetoxy 6-chloroadenosine.
21. A method according to claim 20, wherein triacetoxy inosine is chlorinated to form triacetoxy 6-chloroadenosine.
22. A method according to claim 21, wherein triacetoxy inosine is chlorinated using thionyl chloride or POCl_3 as chlorinating reagent.
23. A method according to any of claims 20 to 22, which further comprises isolating the triacetoxy 6-chloroadenosine produced.

24. A method according to any of claims 20 to 23, which further comprises converting inosine to triacetoxy inosine.
25. A method according to claim 24, wherein inosine is acetylated to form triacetoxy inosine.
26. A method according to claim 25, wherein inosine is acetylated using acetic anhydride as acetylating reagent.
27. A method according to any of claims 24 to 26, which further comprises isolating the triacetoxy inosine produced.
28. A method of synthesis of spongiosine which comprises the steps shown in scheme 1.
29. A method of synthesis of spongiosine which is substantially as described with reference to steps 1 to 5 of the Example.
30. A 2-substituted adenosine of formula I synthesized by a method according to any of claims 1-9, or 11-29.
31. A method of synthesis of 2,6-dimethoxy adenosine which is substantially as described with reference to steps 1 to 4 of the Example.
32. A compound of formula II synthesized by a method according to any of claims 10 to 27, or 31.
33. Use of a compound of formula II, triacetoxy 2-nitro, 6-chloroadenosine, triacetoxy 6-chloroadenosine, triacetoxy inosine, or inosine in the synthesis of a compound of formula I.

34. Use of triacetoxy 2-nitro, 6-chloroadenosine, triacetoxy 6-chloroadenosine, triacetoxy inosine, or inosine in the synthesis of a compound of formula II.
35. A method of producing a nitrated substituted adenosine which comprises nitrating a substituted adenosine using TBAN or TMAN, and reducing the amount of TBA or TMA impurity contaminating the nitrated substituted adenosine.
36. A method according to claim 35, wherein the amount of TBA or TMA impurity is reduced by triturating the nitrated substituted adenosine from isopropanol or ethanol, and washing the triturated product with a mixture of water and ethanol.